Appendix B

Candidate Methods

Appendix B CANDIDATE METHODS

This section includes the complete information on the key program elements (i.e., habitat selection, sampling gear, sampling method, area sampled, replication, subsampling and enumeration, taxonomic identification, quality assurance procedures, data analysis/metrics, habitat assessment, and purpose for monitoring), which is summarized in Chapter 3.

California Department of Fish and Game - Aquatic Bioassessment Laboratory

DFG was the first water resource agency to be asked to assess the condition of a freshwater stream using the U.S. EPA's Rapid Bioassessment Procedure (RBPs) (Plafkin *et al.* 1989). The Lahontan Board requested the assessment in 1993 as part of the NPDES requirement of the DFG Hot Creek Hatchery in Mono County. The request necessitated the need to adapt the RBPs to California and the resulting protocol became the California Stream Bioassessment Procedure (CSBP). Because the CSBP was developed for a point-source assessment, it incorporated the use of replicated sampling of a single, richest habitat. Although not consistent with the RBP, DFG decided on this procedure for the following reasons: a) the immediate need for bioassessment was for point-source assessments, enforcements and diagnosis of known, but undocumented water quality impairment; b) there was no interest, at that time, in using bioassessment as an ambient monitoring tool; and c) the ability to produce a measure of biological metric variability at every monitoring site was deemed necessary to convince water resource managers of the robustness of biological assessments.

The CSBP is a regional adaptation of the U.S. Environmental Protection Agency (EPA) Rapid Bioassessment Protocols (Barbour *et al.* 1999). The CSBP was reviewed and refined by a CABW workgroup in 1994 and 1995 resulting in an updated version in 1996. The CSBP for wadeable streams and rivers has remained consistent over the years and is recognized by the U.S. EPA as California's standardized bioassessment procedure (Davis et al. 1996). Since 1993, the ABL has processed nearly 9000 samples collected using the CSBP at more than 2500 sites throughout California. Thousands of additional CSBP samples have been collected and processed by other entities. In addition to the CSBP for wadeable streams and rivers, as of 2002, there are versions of the CSBP for non-wadeable streams (draft), citizen monitors, lentic environments (California Lentic Bioassessment Procedure), and there is also a modification of the CSBP in which samples are composited for sites that are part of an ambient bioassessment program (this CSBP modification has been adopted by the Nevada DEQ).

1) *Habitat selection:* Riffle habitat is the only habitat sampled using this method. A stream reach is chosen that contains at least five riffles within the same order and relative gradient. If no riffles are present, or less than five within a reasonable distance, the reach is determined as 40 times the wetted width with a minimum reach length of 150 m and a maximum length of 500 m.

2) *Sampling gear:* All samples are collected using a D-frame kicknet with 500 µm mesh netting.

3) *Sampling method:* CSBP utilizes separate point and non-point source sampling designs when conducting ambient bioassessments. When sampling for point source discharges, at least one riffle in the unaffected upstream portion of the reach and one or more riffles in the affected portion of the reach are sampled; one sample is collected from three randomly chosen transects in each riffle. On the other hand, when sampling for non-point source discharges, one sample is collected from the upstream third of 3 randomly chosen riffles.

   <u>Point Source Design</u>

   Step 1. A measuring tape is placed along the bank of the entire riffle selected. Each meter or 3 foot mark represents a possible transect location. Three transects perpendicular to the flow are selected from all possible meter marks along the measuring tape using a random number table.

   Step 2. Three locations are chosen along the transect where the samples are to be collected. If the substrate is fairly similar and there is no structure along the transect, the three locations will be on the side margins and the center of the stream. If there is substrate and structure complexity along the transect, the three locations are selected to best reflect it.

   Step 3. Starting downstream, collections are made by placing the D-frame kick-net onto the substrate and disturbing a one by two foot portion of substrate upstream of the kick-net to approximately 4-6 inches in depth. Large rocks are scrubbed by hand under water in front of the net. A consistent sampling effort (approximately one to three minutes) is maintained at each site. The 3 collections within the transect are combined to make one "composite" sample.

   Step 4. The contents of the kick-net are placed in a standard size 35 sieve (0.5 mm mesh) or white enameled tray. The larger twigs, leaves and rocks are removed by hand after carefully inspecting for clinging organisms. The sampled material and label are placed in a jar and completely fill with 95% ethanol.

   Step 5. Proceeding upstream, repeat Steps 2 and 3 for the next two randomly chosen transects within the riffle.

   <u>Non-point Source Design</u>

   Step 1. Three of the five riffles within the selected reach are randomly chosen using a random number table.

   Step 2. A measuring tape is placed along the bank of the entire riffle selected. One transect is selected from all possible meter marks along the top third of the riffle using a random number table.

   Steps 3-6. Follow steps 2-5 for point source sampling.

4) *Area sampled:* The total area sampled per composite sample, or transect, is 0.54 m^2. Since there are 3 transects sampled per site, the total area sampled at each site is 1.62 m^2.

5) *Replication:* Three replicate composite samples are collected from each site.

6) *Subsampling and enumeration:*

Step 1. The contents of the sample jar is emptied into the # 35 sieve (0.5 mm mesh) and thoroughly rinsed with water.

Step 2. Once the sample is rinsed, debris larger than 2 inch is removed. Green leaves, twigs and rocks are also discarded.

Step 3. The cleaned material is placed into a plastic tray marked with equally sized, numbered grids (approximately two by two inches). Do not allow any excess water into the tray. The moist, cleaned debris is spread on the bottom of the tray using as many grids necessary to obtain an approximate thickness of 2 inch.

Step 4. Randomly chosen grids are removed and sorted until 300 macroinvertebrates are counted. The specimens are placed in a clean petri dish containing 70% ethanol/5% glycerin. The remaining organisms in the last grid are counted but are not included with the 300 used for identification.

7) *Taxonomic identification:* 300 specimens from each sample are identified to the standardized level (genus and/or species) using appropriate taxonomic keys. Identified specimens are placed in individual glass vials for each taxon. Each vial contains a label with taxonomic name, bioassessment laboratory number, stream, county, collection date and collector's name. The voucher collection is labeled and returned to the Sample Depository.

8) Quality assurance procedures:

QA for Collecting Macroinvertebrate Samples

The following procedures are implemented to help field crews collect unbiased and consistent macroinvertebrate samples:

1. Most sampling reaches should contain riffles that are at least 10 meters long, one meter wide and have a homogenous gravel/cobble substrate with swift water velocity. However, there are approved modifications of the CSBP when these conditions do not exist.
2. A DFG biologist or project supervisor trains all field crews in the use of the macroinvertebrate sampling procedures described in the CSBP. Field personnel are to review the CSBPs before each field season.
3. During the training, crew members practice collecting BMI samples as described in the CSBP. The 2 ft^2 area upstream of the sampling device is delineated using the measuring tape or a metal grid and the collection effort is timed. The method is practiced repeatedly until each crew member has demonstrated sampling consistency. Throughout the sampling season, sampling effort is timed and sampled area is measured for approximately 20% of the sampling events.

QA for Measuring Physical/Habitat Quality

The following procedures will help to standardize individual observations to reduce differences in scores:

1. A DFG biologist or a project supervisor trains field crews in the use of the EPA physical/habitat assessment procedures. Field personnel are to review these procedures before each field season.
2. At the beginning of each field season, all crew members are to conduct a physical/habitat assessment of two practice stream reaches. The first stream reach is assessed as a team and each of the 10 physical/habitat parameters described in the EPA

procedure is discussed in detail. The second stream reach is assessed individually and when members are finished, the 10 parameters are discussed and discrepancies are resolved.

3.Crews or individuals assessing physical/habitat quality are to frequently mix personnel or alternate assessment responsibilities. At the end of each field day, crew members are to discuss habitat assessment results and resolve discrepancies.

4.The Project Supervisor randomly pre-selects 10 - 20% of the stream reaches where each crew member will be asked to assess the physical/habitat parameters separately. The discrepancies in individual crew member scores should be discussed and resolved with the Project Supervisor.

QA for the Laboratory

The CSBP uses the following procedures in the bioassessment laboratory to ensure that quality data is produced:

Subsampling - The Subsampling Technician systematically transfers organisms from the sample to a collection vial then transfers the processed sample debris (remnant) into a Remnant jar. At least 10% of the Remnant samples are examined by the QA Taxonomist for organisms that may have been overlooked during subsampling. For subsamples containing 300 or more organisms, the Remnant sample should contain fewer than 10% of the total organisms subsampled. The Remnant for samples containing fewer than 300 organisms should contain fewer than 30 organisms.

Taxonomic Identification and Enumeration - The QA Taxonomist checks at least 10% of the samples for taxonomic accuracy and enumeration of individuals within each taxon. The same sample numbers that were selected randomly for the subsampling quality control should be used for this procedure. Misidentifications and/or taxonomic discrepancies as well as enumeration errors are noted on the laboratory benchsheets. The Laboratory Supervisor determines if the errors warrant corrective action.

Organism Recovery - During the sorting and identification process organisms may be lost, miscounted or discarded. Taxonomists will record the number of organisms discarded and a justification for discarding on the laboratory benchsheets. Organisms may be discarded for several reasons including: 1) subsampler mistakes (e.g. inclusion of terrestrial or semi-aquatic organisms or exuviae), 2) small size (< 0.5 mm), 3) poor condition or 4) fragments of organisms. The number of organisms recovered at the end of sample processing is recorded and a percent recovery determined for all samples. Concern is warranted when organism recoveries fall below 90%. Samples with recoveries below 90% are checked for counting errors and laboratory benchsheets are checked to determine the number of discarded organisms. If the number of discarded organisms is high, then the technician that performed the subsampling is informed and re-trained if necessary.

Corrective Action - Any quality control parameter that is considered out of range is followed by a standard corrective action that includes two levels. Level I corrective action includes an investigation for the source of error or discrepancy derived from the quality control parameter. Level II corrective action includes checking all samples for the error derived from the quality control parameter but is initiated only after the results of the Level I process justify it. The decision to initiate Level II corrective action and reanalyze samples or conduct quality control on additional samples is made by the Laboratory Supervisor.

Interlaboratory Taxonomic Validation - An external laboratory or taxonomic specialist is consulted on a regular basis to verify taxonomic accuracy. External validation can be performed on selected taxa to help the laboratory taxonomists with problem groups of BMIs and to verify representative specimens of all taxa assembled in a reference collection.

Bioassessment Validation - The CSBP recommends at least 10% bioassessment validation where whole samples of 300 identified specimens are randomly selected from all samples either for a particular project or for all samples processed within a set time period such as each 6 months or a year. The labels are removed from the vials and replaced with a coded label that does not show the taxonomic name of the specimens. The validation laboratory or specialist is to identify and enumerate all specimens in each vial and produce a taxonomic list. There will inevitably be some disagreements between the bioassessment and the external laboratory on taxonomic identification. These taxa should be re-examined by both parties and a resolution reached before a final QA report is written.

9) *Data analysis/Metrics:* The CSBP analysis procedures are based on the EPA=s multi-metric approach to bioassessment data analysis. A taxonomic list of the macroinvertebrates identified in each sample is generated for each project along with a table of sample values and means for the biological metrics listed in the table below. Variability of the sample values are expressed as the CV. Significance testing is used for point source sampling programs and ranking procedures are used to compare sites sampled using the non-point sampling design.

10) *Habitat assessment:* Physical/habitat parameters are assessed using a ranking system ranging from optimal to poor condition. This rapid ranking system is derived from the procedures outline in the "Revised Rapid Bioassessment Protocols for use in Streams and Rivers" (Barbour et al. 1999), and relies on visual evaluation and is inherently subjective. The following ten parameters are evaluated and ranked: 1) epifaunal substrate/available cover, 2) embeddedness, 3) velocity/depth regimes, 4) sediment deposition, 5) channel flow status, 6) channel alteration, 7) frequency of riffles (or bends), 8) bank stability, 9) vegetative protection, 10) riparian vegetative zone width. In addition to EPA RBP habitat measures, the CSBP also evaluates measures cover, quantitative substrate, pebble count, substrate consolidation, depth and width, and velocity.

11) Purpose for monitoring:

- Enforcement and resource damage assessment
- Use attainability
- Ambient monitoring
- Special studies and research

United States Forest Service Pacific Southwest Region (California) Bioassessment Program

The US Forest Service uses a method developed at Utah State University by Charles Hawkins, Jeff Ostermiller, and Mark Vinson. The invertebrate protocols were modified from the designs used by the states of Oregon and Washington and the Bureau of Land Management's National Monitoring Center.

1) *Habitat selection:* Sampling is done at the first fast-water (e.g., riffles, runs) habitat encountered at the site and will continue upstream for the next three fast-water habitat units. If no fast-water habitats occur, eight constant area samples are taken from shallow, slow-water habitat units.

2) *Sampling gear:* All samples are collected using a Surber sampler ($0.09m^2$) with 500 µm mesh netting and a one meter long net to prevent backwashing.

3) *Sampling method:* Two types of samples are collected at each site: 1) a series of eight fixed area samples taken from four fast-water habitat units and 2) a single 10-minute qualitative sample taken from all major habitat types approximately in proportion to their occurrence.

   Fixed Area Samples
   Net placement within each habitat unit is determined by generating two pairs of random numbers between 0 and 9. The first number in each pair (multiplied by 10) represents the percent upstream along the habitat unit's length. The second number in each pair represents the percent of the stream's width from bank left. This process is repeated to locate the second sampling location. Samples are taken where the length and width distances intersect. If it is not possible to take a sample at one or both of these locations, additional random numbers are drawn. Invertebrates are collected from within the $0.09m^2$ area in front of the sampler starting from the upstream edge of the sampling plot and working downstream. Large stones are rubbed and inspected to ensure that all organisms are dislodge and collected. After removing all large stones, small substrates (i.e., sand or gravel) are disturbed to a depth of approximately 10 cm by raking and stirring until no additional organisms or organic matter is being washed into the net.

   10-Minute Qualitative Samples
   The area is visually appraised and the proportion of different habitat types is estimated. The 10-minute sampling period is apportioned so that each of the habitat types is sampled roughly in proportion to their occurrence.

4) *Area sampled:* The total area sampled per fixed area composite is $0.72m^2$. The total area for the fixed time sample is highly variable.

5) *Replication:* There are no replicate samples collected using this method.

6) *Subsampling and enumeration:* The following is a step-by-step description of how quantitative benthic macroinvertebrate samples are processed:

Step 1. The sample is poured through an appropriately sized 250 µm sieve. If the sample contains a lot of sand and gravel, the organic matter will need to be decanted. The entire sample is then poured from the sieve into a bucket partially filled with water. The bucket is swirled so that the organisms and organic matter become suspended in the water column and the heavier sand and gravel falls to the bottom. The water and floating organisms are carefully decanted back through the sieve. Water is continually added to the bucket and decanted until no organic matter remains in the bucket. When finished, the remaining material in the bucket is closely examined and any caddis flies, snails, clams, or other animals that remain are picked out. These organisms are added to those on the sieve.

Step 2. The sample on the sieve is rinsed under the faucet to wash additional fine particles and silt away.

Step 3. The sieve is then placed in an enamel pan or bucket that is partially filled with water and the sample is "floated" so that it becomes level within the sieve. Once leveled, the sieve is carefully removed from the enamel pan. An appropriately sized separator bar is placed into the sieve to split the material in the sieve in half.

Step 4. A coin is flipped to determine which half of the sample is to be processed (heads = right or top, tails = left or bottom). The portion of the sample to be processed is kept in the sieve, and the other half is transferred into a cup using a spoon or rinsed into the cup using an alcohol filled squeeze bottle. The cup is covered with ParaFilm and the portion or split of the sample is written on the lid, e.g., 50%. If it appears that less than 50% of the sample will be sorted, the sieve is placed back in the enamel pan and the material is re-floated to level it, and repeat the same process described above until it appears that approximately 500 organisms remain in one-half of the sieve. Once a split is started it must be finished to its entirety.

Step 5. The material to be sorted is placed little-by-little into a petri dish and all organisms within the petri dish are removed under a dissecting microscope at 7-20x magnification. As the organisms are removed, they are counted and separated into different taxonomic orders. Some representative individuals of the following groups are removed from the sample but not counted as part of the 500 bugs:

- eggs
- exuviae, molt skins
- adult insects – terrestrial or aquatic
- empty snail shells
- brooding juveniles, e.g., small amphipods
- zooplankton
- Collembola

All worms are put in the non-insect vial, but are not counted as part of the 500 bugs. Additional portions of the sample (splits) are sorted until at least 500 organisms are found. The target is to sort between 500 and 550 bugs. If 600 organisms are exceeded, the entire sample must be redone.

Step 6. When 500 bugs have been removed, the entire sample is spread evenly throughout a large white enamel pan. The pan is systematically searched for 10 minutes, and any organisms that have not been found in the split samples thus far are removed. These bugs are placed into a separate vial labeled "B/R" for "Big/Rare".

7) *Taxonomic identification*: Insects are primarily identified to the genus level, Chironomidae are identified to the sub-family level, and non-insect invertebrates are identified to various levels depending on available keys.

8) *Quality assurance procedures:* Not Avaliable.

9) *Data analysis/Metrics:* No standard data analysis procedure has been designated at this time. RIVPACS will be utilized to develop a model to determine the level of impact to the biological assemblage at the site.

10) *Habitat assessment:* Site evaluations are conducted to determine the suitability of reference sites and the degree or type of degradation occurring within test sites. Three major categories are evaluated: Riparian, bank, and channel.
Riparian – 1) vegetative condition, 2) percent historic floodplain remaining intact, 3) anthropogenic activity within the floodplain, 4) alteration of the vegetation within the floodplain, and 5) erosional deposition into stream from surrounding hillslopes.
Bank – 1) percent of streambank with deep, binding root mass, and 2) percent of stream with active lateral cutting.
Channel – 1) siltation, and 2) large woody debris. Additional measures are taken at each site for channel shade, width, depth, substrate, stream slope, dominant erosional habitat type, and dominant depositional habitat type.

11) *Purpose for monitoring:*
    - Development of biocriteria and bioassessment protocol
    - Monitoring of impacts from timber harvest, grazing and mining activities
    - Ensure compliance with the Clean Water Act
    - TMDL implementation

United States Geologic Survey - National Water Quality Assessment

The USGS National Water Quality Assessment (NAWQA) program uses a benthic macroinvertebrate sampling method developed by Thomas F. Cuffney, Martin E. Gurtz, and Michael R. Meador and revised method for characterizing stream habitat developed by Faith A. Fitzpatrick, Ian R. Waite, Patricia J. D'Arconte, Michael R. Meador, Molly A. Maupin, and Martin E. Gurtz. However, prior to 1998, when most of the California data was collected, NAWQA used a stream habitat assessment method developed by Michael R. Meador, Cliff R. Hupp, Thomas F. Cuffney, and Martin E. Gurtz.

1) *Habitat selection:* Two types of samples are collected at each site: 1) qualitative multi-habitat (QMH) sampling and 2) richest targeted habitat (RTH) sampling. For QMH samples, all habitat types present in the reach are selected. Semi-quantitative RTH sampling focuses on sampling a habitat supporting the faunistically richest community of benthic invertebrates, usually a fast-flowing, coarse-grained riffle. When riffles are not available, woody debris is sampled.

2) *Sampling gear:* The primary sampling gear used to collect QMH samples is a D-frame kick net equipped with a 210 µm mesh net. RTH samples are collected using a 0.5 m by 0.25 m rectangular frame net equipped with a 425 µm mesh net.

3) *Sampling method:* Two types of samples are collected at each site: 1) qualitative multi-habitat sampling (QMH) and 2) richest targeted habitat (RTH) sampling.

   Qualitative Multi-habitat
   QMH sampling effort is variable because it depends on the types of habitats present and their abundance within the sampling reach. A D-frame kick net is used to collect samples by kicking, dipping, or sweeping in a manner appropriate for the instream habitat type being sampled. When possible, equal sampling effort is applied to each habitat type within the sampling reach. This is usually accomplished by dividing the available 1-hour sampling time equally among the instream habitat types. The D-frame kick net collections are supplemented with visual collections and, where appropriate, with seines to collect highly-motile invertebrates. Visual collections involve manually collecting large rocks, coarse organic debris, clay from stream margins, root wads, and macrophytes or other substrates, and visually locating and removing any associated organisms.

   Richest Targeted Habitat
   The rectangular frame net is held perpendicular to the direction of flow and pressed tightly against the stream bottom. Benthic invertebrates are collected from an area of approximately 0.25 m^2 immediately upstream of the net. If 50 percent or more of a rock lies within the sampling area, it is removed and held in front of the net opening, and attached organisms are dislodged into the net by gently brushing the surface of the rock with the hand and then with a fingernail brush. After a rock is brushed, it is examined to determine if any closely adhering organisms are present. Such organisms are removed from the rock surfaces using forceps and placed into a separate vial holding the large-rare sample component. This sample component contains large organisms that can interfere with sample splitting and rare organisms that might be lost during sample splitting. After the large rocks (fist size and larger) are removed, the sampling area is dug to a depth of about 0.1 m. Any remaining organisms are dislodged into the net by kicking the substrate within the sample area for a period of 30 seconds. The material collected in the net is then transferred to an appropriate container, usually a 19-L (5-gal) plastic bucket or dishpan, for further field processing. Subsequent elements of the composite sample are added to this container and then processed, or the separate elements may be processed and then composited. A minimum of five samples, apportioned within and among examples of the targeted instream habitat type, are composited into a single RTH sample. Examples of the targeted habitat type are collected from across the length and width of the sampling reach.

4) *Area sampled:* The total area sampled per RTH composite is 1.25 m^2. The total area sampled for the QTH sample is variable.

5) *Replication:* More intensive sampling is conducted at a subset of four to six sites to assess spatial variability among reaches and short-term temporal variability at a site. At these sites, three sampling reaches are established to represent environmental conditions associated with the basic fixed site. One sampling reach is sampled in each of 3 successive years to estimate short-term temporal variability. Two additional sampling reaches are sampled in 1 year to assess the magnitude of reach-to-reach variability.

6) *Subsampling and enumeration:* Samples are field processed to reduce the volume of each sample component so that it fits in to a 1-L sample container with ample room for preservative. Sample volume reductions are accomplished by removing large debris, elutriating to remove inorganic sediments, and then splitting the elutriated samples. Field processing can result in the production of four sample components from each composite sample: large-rare, main-body, elutriate, and split-sample components.

   Field processing begins with the removal of large rocks and organic debris, such as leaves, twigs, and roots, from the sample. These materials are discarded after all attached invertebrates have been removed. The remaining material is examined for large, rare organisms that can be lost during subsequent sample splitting. These large-rare organisms are removed and placed in a separate, labeled container that is identified as the "large-rare" sample component. All organisms that are picked from the sample by hand prior to sample splitting are added to the large-rare sample component.

   The remaining sample material is elutriated onto an appropriately sized sieve (425-Φm mesh for semi-quantitative samples and 212-Φm mesh for qualitative samples) to separate the lighter organic material from the heavier sand and gravel. Elutriation is usually accomplished by placing the sample in a deep bucket filled about one-fourth to one-half with water. The contents of the bucket are stirred by hand to suspend as much material as possible. The bucket is picked up, swirled, and then gently decanted onto an appropriate sieve. The elutriation process is repeated until it appears that only sand and gravel remain in the elutriation bucket. The sand, gravel, and small pebbles remaining in the bucket are visually examined for invertebrates, particularly case-building caddisflies and small mollusks. Invertebrates that are removed during this process are added to the large-rare sample component. Once free of invertebrates, the left-over sand and gravel is retained as a quality-assurance check on the efficiency of elutriation.

   Elutriated material retained on the sieve is quickly examined for large, rare organisms that are added to the large-rare sample component. If, after elutriation and compositing, the volume of material constituting the main-body or elutriate sample component exceeds 0.75 L, that sample component is split in the field. Any debris or large organisms that remain in the sample is removed to simplify the sample-splitting process. Organisms so removed are added to the large-rare sample component, whereas debris is discarded after any attached invertebrates are removed.

   Sample splitting is accomplished by using either a special sieve sample splitter (Mason, 1991) or a sieve diameter splitting method. Once the sample has been split, one half of the sample is randomly selected. If the sample being processed is an elutriate sample, then the half of the sample selected is retained for analysis and the other half is discarded. If the sample being processed is a main-body sample, then the half of the sample selected is designated as the main-body component and the other half is designated as the "split" sample component. Some particularly large samples may require repeated splitting to obtain suitable volumes (less than or equal to 0.75 L) of main-body, split, and elutriate sample components. If the resulting split-sample component (elutriate, split, or main-body) exceeds 0.75 L, it is split again. Careful records of the number of splits performed and the portion of the original sample retained for analysis are kept and entered on the appropriate field data sheet.

After samples have been processed, they are transferred to appropriately sized plastic sample containers and an internal sample label is filled out and placed in the container. The sample should occupy approximately one-half to three-fourths of the container volume. A solution of 10% buffered formalin is added to bring the total volume to within 2 cm of the top of the jar. The jar is then capped and slowly inverted several times to mix the contents of the jar with the formalin solution and to remove any air trapped in the sample matrix. The jar is then opened and topped off with 10% buffered formalin.

<u>Qualitative Visual Sort Method</u>
The preservative is rinsed from the sample through a sieve that has a mesh size less than or equal to that used in the field. If necessary, the sample is elutriated to separate inorganic and organic detritus. The sample is then size-fractionated by using a 4.75-mm sieve. To ensure consistent and effective sorting, the sample is apportioned evenly among multiple white sorting trays. The number and size of the trays are adjusted so that about 50 percent of the bottom is visible in each tray. Total sorting time is limited to 2 hours. The coarse-size fraction is sorted for about 0.25 hour. The remaining time, about 1.75 hours, is apportioned between the fine-size fraction and any elutriated inorganic debris; however, if the taxonomist determines that the entire sample has been adequately sorted without adding different taxa, and then sorting is terminated at less than 2 hours. This action is approved by a second taxonomist and noted on the bench data sheet. If the volume of the fine-size fraction is such that it cannot be adequately sorted in about 1.75 hours, then the sample is divided directly on a sieve or on an appropriate sub-sampling frame so that at least 25 percent of this fine-size fraction can be sorted. The remaining unsorted remnant is quickly scanned and sorted for distinct taxa.

Each tray is sorted systematically by a taxonomist for mature, undamaged organisms. After one complete pass of the tray, the detritus is redistributed by rocking the tray and sorting continues. BMIs are sorted into gross taxonomic categories and placed into polyseal screw-cap vials that contain 70% ethanol. At least 50 Chironomidae larvae are sorted whenever possible. Visually distinguishing Genus- or Species-level diversity for some BMI taxa is often difficult; therefore, comparable numbers of organisms of these groups are sorted from each tray of each sample. All unique mollusk shells are sorted, even if the body of the organism is not present.

<u>Quantitative Fixed-Count Subsampling Method</u>
The principal objective of the fixed-count method is to identify and estimate the abundance of each BMI taxon sorted from the sample. This method is similar to the USEPA's RBP sample-processing procedure (Barbour et al. 1999; Plafkin et al. 1989).

The fixed count is based on a minimum number of organisms sorted from the sample and is defined by the study's data quality objectives (for example, 100-, 200-, or 300-organism fixed-count target).

Samples containing more organisms than the fixed-count target are subsampled by using a subsampling frame partitioned into 5.1- by 5.1-cm grids. However, uniformly distributing a sample in a subsampling frame is often difficult, and organisms in the sample matrix tend to have a clumped distribution. Therefore, subsampling by simply acquiring a single, very small portion from a subsampling frame could lead to extreme errors in estimating the abundance of taxa in the sample. The method described below uses multiple, randomly selected 5.1- by 5.1-cm portions of the original sample (stage-1 grids) to estimate abundance accurately. Large-rare organisms are sorted from any remaining portion(s) of the sample after the random subsampling is complete.

Total sorting time is limited up to a maximum of 8 hours, depending on the fixed-count target. The time limitation has been implemented to avoid spending too much time on samples that contain few or have exceedingly difficult detritus to sort. A generalized processing procedure is listed as follows:

- The sample is uniformly distributed in a subsampling frame (stage-1 subsampling frame).
- An estimate of the average number of organisms per stage-1 grid is obtained.
- By using the average number of organisms per stage-1 grid, an appropriate processing strategy is selected.
- The grids are randomly selected from either a stage-1 or a stage-2 subsampling frame, and organisms are sorted from each grid.
- Large-rare organisms are sorted from any remaining unsorted portion(s) of the sample.

Three sizes of gridded subsampling frames are used, 12 grid (15.2 cm X 20.3 cm X 3.8 cm), 24 grid (20.3 cm X 30.5 cm X 3.8 cm), and 42 grid (30.5 cm X 35.6 cm X 3.8 cm). The size of the subsampling frame chosen depends on the total sample volume and organism density; frame size increases with sample volume and density. If the volume of a sample is very low but the density of the BMIs is high, the subsampling frame size is dictated by the density of organisms in the sample. Occasionally, the volume of detritus is so small and the BMIs are so depauperate that the use of a sub-sampling frame is not necessary. The primary objective is to choose a frame size for uniform dispersal of the sample.

The mean number of organisms per stage-1 grid is used to determine the appropriate subsampling strategy. This mean is obtained by randomly selecting five grids from the stage-1 subsampling frame and uniformly distributing the material from each grid into separate, appropriately sized, estimation trays. Estimation trays with either 49 or 81 grids can be used to obtain a uniform distribution and density of sample material. The organisms in each of three randomly chosen estimation tray grids are counted and used to estimate the number of organisms in each estimation tray and, hence, each stage-1 grid. Separate estimates are made from each of the five estimation trays. The resulting five estimates are averaged to give an estimate of the number of organisms in each stage-1 grid. An informed processing decision can be made once the mean number of organisms per stage-1grid has been estimated. Sub-sampling may involve processing multiple randomly selected stage-1 grids from the stage-1 subsampling frame (1-stage sub-sampling) or a further subsampling of three to five stage-1 grids (2-stage subsampling). Numeric criteria are used to determine the appropriate subsampling strategy. Once the appropriate level of subsampling has been achieved, the approximate number of random grids are randomly selected for sorting. Additional grids are randomly selected as needed to reach the fixed-count target.

The contents of each randomly chosen stage-1 or stage-2 grid are sorted separately by using a dissecting microscope with X 10 magnification. All identifiable organisms are sorted. Mollusk shells are only sorted if the animals are present in the shells. Only a portion of colonial organisms, such as Bryozoa or Porifera, is sorted to document its presence in the sample. Vertebrates, exuviae, invertebrate eggs, microcrustaceans, and terrestrial organisms are not sorted. However, terrestrial insects that have an aquatic lifestage are sorted.

Once sorting has begun, the grid is sorted to completion even if numeric or time frame criteria are exceeded. Organisms are enumerated as they are removed from each grid and pre-sorted into categories. Organisms are placed in polyseal capped vials containing 70% ethanol. The sort-time criteria, excluding time required to prepare the sample and estimate grid densities, are 8 hours for a 300-organism fixed-count target and 3 hours for a 100-organism fixed-count target.

Some large-rare taxa may be present but at such low densities that it is unlikely that they will be encountered in the random subsamples. The quantitative sample-processing method accounts for these large-rare taxa by visually sorting them from the unsorted portion of the sample. This sorting is limited to 15 minutes. If inorganic debris is separated from the sample, this debris also is sorted for large-rare organisms.

7) *Taxonomic identification*: The National Water Quality Laboratory (NWQL) Biological Group (BG) provides three levels of taxonomic assessment for BMI samples. These levels include (1) the Standard Taxonomic Assessment (STA), (2) the Rapid Taxonomic Assessment (RTA), and (3) the Custom Taxonomic Assessment (CTA). Each provides a different basic level of taxonomic resolution to address various water-quality and related data-analysis objectives. The STA and RTA are adapted from the U.S. Environmental Protection Agency (USEPA) Rapid Bioassessment Protocols (RBP) (Barbour et al., 1999; Plafkin et al., 1989). The STA represents a taxonomic effort similar to that described in the USEPA RBP III (Barbour et al., 1999; Plafkin et al., 1989) and in many other state biomonitoring protocols. It is currently (2000) the level of resolution used by the USGS NAWQA Program for BMI samples. In general, mollusks, crustaceans and insects are identified to either the Genus or Species level. Aquatic worms are identified to the Family level. Other BMI groups, such as flatworms and nematodes, are typically identified at higher taxonomic levels (for example, Phylum or Class). The RTA represents a taxonomic effort similar to the USEPA RBP II (Barbour et al., 1999; Plafkin et al., 1989). In general, all BMI groups are identified to the Family level, except for

groups such as flatworms and nematodes, which are typically identified at higher taxonomic levels (for example, Phylum or Class). The CTA provides a customer-specified taxonomic effort that is not provided in the STA or RTA.

8) *Quality assurance procedures:* Not available.

9) *Data analysis/Metrics:* Not available.

10) *Habitat assessment:* Habitat is assessed using a first-level reach characterization and a more detailed second-level reach characterization.

First-level reach characterization:

Six transects, as a minimum, are established to collect information throughout the reach with two transects established at or near each boundary. If the reach is established on the basis of the presence of two examples of each of two types of geomorphic channel units, the remaining four transects are established at the middle of each geomorphic channel unit. If the reach is defined on the basis of channel width, then the remaining four transects are evenly spaced throughout the reach. Transects are oriented perpendicular to streamflow.

- Channel width: Measure the channel width along the transect from left edge of water to right edge of water.
- Bank width: Bank width is the distance between the channel bed and the flood plain. This distance is measured with a tape measure or rangefinder.
- Flood-plain width: Flood-plain width is measured as the distance between the significant changes in slope that distinguish the flood plain from terraces and riparian features. If this distance is less than 50 m, it can be measured with a tape measure or rangefinder. However, if the flood-plain width is greater than 50 m, it is determined from maps or aerial photographs, and indicated as greater than 50 m on the form.

For the next 3 items, data are collected at three points along each transect. These points should correspond to the thalweg, and to two locations that are equally spaced along the transect (or three equally spaced locations if no thalweg is apparent).

- Depth: In wadeable reaches, water depth between the water surface and the bed substrate is measured with a wading rod and recorded. In nonwadeable reaches, a sounding line or hydroacoustic depth meter may be necessary to determine depth. When using a hydroacoustic depth meter, the investigator maneuvers the boat along the transect with the meter operating, so as to produce a continuous recording of water depth along the transect. Three depth measurements, one at the thalweg and two at locations equally spaced along the transect, can be determined from the hydroacoustic chart.
- Velocity: In wadeable reaches, record velocity using a Price AA current meter, pygmy meter, or Gurley meter. In nonwadeable reaches, use a velocity meter appropriate for velocity determinations at that site. Velocity is recorded at 60% depth

where depth is less than 1 m. At depths greater than or equal to 1 m, two velocity measurements, one at 20% depth and the other at 80% depth, are recorded.

- Bed substrate: Determine the spatially dominant and subdominant substrates. In turbid wadeable reaches and in nonwadeable reaches, a sample of the substrate is obtained by using an appropriate device such as a shovel, Ponar sampler, or Ekman dredge. In turbid wadeable reaches and in nonwadeable reaches, the presence of boulders and bedrock cannot be determined by sampling. However, in turbid wadeable reaches, the presence of these substrate types can be determined by touch. In nonwadeable reaches where sampling devices cannot yield a substrate sample, acoustic recording of the stream bottom along the transect can detect boulders and bedrock.

- Embeddedness: Embeddedness is measured by rating the percentage of the surface area of the larger-sized particles (by visual estimation) covered by fine sediment. To determine how much of the surface area of large particles is covered in order to provide a rating, select five relatively large (gravel to boulder size) substrate particles at the three sampling points along the transect and examine them on the sides. Note the percentage of each particle's height that was buried in sediment by the extent of discoloration on the particle. The rating is based on the percentage of coverage of fine sediment as determined from the average percentage of coverage for the five particles. In turbid wadeable reaches and in nonwadeable reaches, a sample of the substrate is obtained using an appropriate device such as a shovel, Ponar sampler, or Ekman dredge.

- Canopy angle: From the midpoint of the transect, use a clinometer to determine the angle from the line of sight of the investigator to the tallest structure (for example, tree, shrub, building, or grass) on the left bank (in the general area of the transect). The same procedure is done at the right bank. The sum of these angles is computed and subtracted from 180 degrees.

- Aspect: Record the aspect (0 to 360 degrees) of the downstream flow of the stream using a compass. At the midpoint of the transect, face downstream and point a compass parallel to streamflow.

- Habitat features: Determine the type and amount (two-dimensional area) of all habitat features that are partly or wholly within a 2-m zone on either side of the transect. Habitat features consist of any mineral or organic matter that produces shelter for aquatic organisms to rest, hide, or feed, and include natural features of a stream such as large boulders, woody debris, undercut banks, and aquatic macrophyte beds, as well as artificial structures such as discarded tires, appliances, and parts of automobiles. Habitat features are not counted when they are in insufficient depth (usually less than 20 cm).

- Bar/Shelf/Island: If channel bars, shelves, or islands are present, measure width using a tape measure or rangefinder. Determine the spatially dominant and subdominant substrates along the transect for the bars, shelves, and islands that occur. Also estimate the percentage of coverage of woody and herbaceous vegetation for the entire bar/shelf/island.

- Bank angle: A clinometer is used to measure the angle formed by the downward-sloping bank as it meets the stream bottom. The angle is determined directly from a clinometer placed on top of a surveyor's rod or meter stick that is aligned parallel to the bank along the transect. The clinometer reading is subtracted from 180 degrees to produce the bank angle. If the height and shape of the bank are such that more than one angle is produced, then an average of three readings is recorded. Both left bank and right bank (facing downstream) angles are recorded.

- Bank height: Determine the left and right distance from the channel bed to the top of the bank. A surveyor's rod and hand level can be used if this distance can be measured directly. If the bank height cannot be measured directly, then it can be estimated. Note that the bottom of the bank is the deepest part of the channel. At large, nonwadeable reaches, topographic maps may be useful in determining bank height.

- Bank vegetation stability: Bank vegetation stability is evaluated using a rating based on four classes that represent percent coverage of the bank surface. The rating includes only that part of the bank that is within 2 m of either side of the transect, to the top of the bank.

- Bank shape: Record the shape of the left and right banks as: concave upward, linear, or convex upward.

- Bank erosion: The types of bank material movement, if present, are noted. These types include mass wasting (debris avalanche, rotational failure, and slab failure), and cut-bank scalloping. Indicate the presence of bank erosion for the left and right banks as: debris avalanche, rotational failure, slab failure, cut-bank scalloping, or none.

- Bank substrate: Determine the spatially dominant and subdominant substrate types that are present in an area of the bank that is within 2 m of either side of the transect, to the top of the bank. This procedure is done for the left and right banks.

- Bank woody vegetation: The point-centered quarter method is used to evaluate density and dominance of bank woody vegetation (Mueller-Dombois and Ellenberg, 1974). Sampling points are established on both banks at the ends of the transect so as to include dominant bank woody vegetation. Four quarters are established at a sampling point at the intersection of two perpendicular lines, one of which is the transect. Trees and shrubs are included in the analysis. Trees are distinguished from shrubs in that trees are at least 2 m high and have a diameter at breast height (dbh) of at least 3 cm. The sampled trees or shrubs are identified to species, and the distance from the sampling point to the nearest tree or shrub in each quarter is measured, along with its dbh. Where bank woody vegetation is growing in narrow strips or rows, the two closest trees or shrubs on either side of the sampling point are measured. Where a single tree or shrub has developed many separate trunks, an average dbh for three trunks is recorded, along with the total number of trunks.

- Photodocumentation: Stream conditions at three transects, including the transects at or near the reach boundaries and one transect representative of reach conditions, are photographed. Semipermanent markers are established at these locations to facilitate taking repeat photographs. Color photographs, preferably slides, are taken that include upstream, transect, and downstream views of the channel and should include a scale reference in the image. The inclination and aspect of the camera lens are important and are measured with a compass. A level camera is preferred to an inclined one because inclination complicates the perspective of the view and makes accurate duplication of repeat photographs difficult. The aspect of the camera is noted by pointing a compass at the central aiming point in the view and recording the compass reading. Photographs are taken facing upstream, facing perpendicular to the channel, and facing downstream, from either the left or right banks.

- Diagrammatic mapping: Draw a schematic or representative map of the reach. The map should include location of geomorphic channel units, habitat features, and bank and flood-plain land use. Indicate the stream type and general shape of the channel.

- Aquatic and riparian vegetation species: Record the species name of all common aquatic (submerged, emergent, and floating) and riparian (bank--herbaceous and woody, and flood plain--herbaceous and woody) species. Be sure to note the five most common for each category.

<u>Second-level reach characterization</u>
A second-level reach characterization also is conducted at all fixed sites. This is a detailed reach characterization and is designed to provide additional quantitative data on geomorphic and hydraulic properties that are critical to the evaluation of temporal changes in the environmental setting and stream habitat. The second-level reach characterization consists of an analysis of hydraulic properties and channel geometry plus additional components tailored to enhance an understanding of temporal changes. The analysis of channel geometry consists of longitudinal profiles of the water surface, flood plain, and channel bed; cross-sectional surveys with levels; a map of the reach; and a quantitative analysis of bed and bank materials. Additional suggested components of the second-level reach characterization include permanent plot vegetation analysis and detailed quantitative mapping of habitat features throughout the reach. Study unit personnel are responsible for developing an appropriate form for recording the second-level reach characterization.

The longitudinal profile of the channel bed is conducted along the thalweg (or the approximate center of the channel if a thalweg is not apparent) on the basis of channel-bed elevations recorded at intervals equal to one channel width. This distance is generally sufficient to determine the mean slope of the reach. The water-surface profile can be determined simultaneously by having the rodman record the water depth at each location and add this value to the channel-bed elevation. Profiles of the flood plain along both banks also are conducted. In nonwadeable reaches, longitudinal profiles of the channel bed are determined using a hydroacoustic depth meter, and water-surface elevations are determined along one bank or both banks.

At a minimum of three locations (both reach boundaries and a location that includes a prominent geomorphic feature), leveled cross-sectional surveys are conducted from left flood plain to right flood plain. Each cross-sectional survey is plotted, with elevation recorded on the ordinate axis and distance in meters along the abscissa. All surveys are conducted in relation to the reference location. A map of the reach is constructed, indicating the locations of the longitudinal profiles and the cross-sect ional surveys. Cross-sectional surveys of nonwadeable reaches include as much information as can possibly be recorded.

In addition to an analysis of channel geometry, a quantitative analysis of channel substrate particle size is conducted. Pebble counts are conducted to determine bed material particle-size distribution in wadeable reaches. At the three surveyed cross sections, a pebble-count transect is established, and the pebble count is conducted in the following method:

(1) Begin the count at each transect at bankfull elevation on the left bank and proceed to bankfull elevation on the right bank.
(2) Proceed one step at a time, with each step constituting a sampling point.
(3) At each step, reach down to the tip of your boot and, with your finger extended, pick up the first pebble-size particle touched by the extended finger.
(4) To reduce sampling bias, look across and not down at the channel bottom when taking steps or retrieving bed material.
(5) As you retrieve each particle, measure the intermediate axis. If the intermediate axis cannot be determined easily, measure the long diameter and the short diameter of the particle, and determine the average of the two numbers.

Thus, the size distribution of particles is determined and expressed in percentage by number of particles. A count of 100 particles is recommended; however, to determine percentages of particle sizes, 50 or 25 particles can be measured. To obtain a quantitative determination of finer grained bed material, three samples of the bed material are collected along each transect and composited. In addition, samples of the bank substrate material can be collected from one bank or both banks. These samples are returned to the laboratory for sieve analysis.

Permanent plot vegetation analysis is also suggested as a component of the second- level reach characterization. To construct a permanent vegetation plot, select an area at the end of each of the surveyed cross sections. A 20- by 20-m plot is identified by using a tape measure to determine the appropriate distance and a compass to establish 90-degree angles at the corners of the plot. The corners are then marked with semipermanent boundary markers. The edge of the plot nearest the bank edge should be at least several meters from the bank. Sample the vegetation by determining the diameter and species of all trees and shrubs within the plot. Record only living trees and shrubs. If the riparian zone is narrow such that a 20- by 20-m plot cannot be established, then two or more smaller plots are established so that the total area sampled equals 400 m^2. Where herbaceous vegetation is clearly dominant, then a 10- by 10-m square plot is established. At herbaceous vegetation plots, the aerial coverage of up to five species is measured, and the percent coverage of these species within the plot is calculated.

Mapping of all geomorphic channel units and habitat features can also provide critical information needed to evaluate temporal trends in habitat. Though the diagrammatic stream map should indicate the presence of these units and features to approximate scale, the first-level reach characterization does not attempt to quantify the occurrence of all features throughout the reach. In the second-level reach characterization, the two-dimensional area of all significant geomorphic channel units and habitat features is determined.

11) *Purpose for monitoring:*

- Describe current water-quality conditions for a large part of the Nation's freshwater streams.
- Describe how water quality is changing over time, and
- Improve our understanding of the primary natural and human factors affecting water quality.

United States Environmental Protection Agency - Environmental Monitoring and Assessment Program

EMAP is a research program to develop the tools necessary to monitor and assess the status and trends of national ecological resources. EMAP's goal is to develop the scientific understanding for translating environmental monitoring data from multiple spatial and temporal scales into assessments of ecological condition and forecasts of the future risks to the sustainability of our natural resources. The objectives of REMAP are to: 1) evaluate and improve EMAP concepts for state and local use, 2) assess the applicability of EMAP indicators at differing spatial scales, and 3) demonstrate the utility of EMAP for resolving issues of importance to EPA Regions and states.

A Regional-EMAP (REMAP) study was conducted in 1994-1995 in Californina's Central Valley, which comprises more than 48,000 miles of surface water and 16 percent of the land area in the State and is one of the nation's most productive agricultural areas. The Central Valley REMAP Project was initiated to assess the biological integrity of agriculture-dominated waterbodies located throughout California's Central Valley. Moreover, USEPA is currently collecting additional bioassessment data in California as part of the EMAP Western Surface Water pilot study, which is a five-year research and monitoring project to assess the ecological condition of streams and rivers across the Western U.S.

Typically, EMAP and REMAP studies use the same sampling methods; however, the Central Valley REMAP study used an earlier method developed by Philip A. Lewis and Donald J. Klemm (see Klemm and Lazorchak 1995), while the Western EMAP study uses a revised method developed by D. J. Klemm, J.M. Lazorchak, and P.A. Lewis (see Lazorchak et al.1998). Only the revised (current) method will be discussed in this section.

1) *Habitat selection:* Each sampling reach is determined as 40 times the wetted width, with a minimum reach length of 150 m and a maximum length of 500 m. The habitats that are sampled are selected randomly by dividing the reach into 11 equidistant cross-sectional

transects, and randomly sampling at the left third, center, or right third from the interior nine transects. For each reach, riffle and run habitat samples are composited into a single "Riffle" sample whereas pool and glide samples are composited into a single "Pool" sample.

2) *Sampling gear:* The primary sampling gear used to collect samples is a modified 0.5 m by 0.3 m rectangular frame kick net equipped with a 595/600 µm mesh net.

3) *Sampling method:* As mentioned previously, the sampling reach is equally divided into 11 cross-sectional transects. At each of the nine interior transects, a sampling point (left, center, or right) is assigned. Once the first sampling point is randomly chosen, points at successive transects are assigned in order (left, center, right). Habitat type is sampled roughly in proportion to their occurrence.

4) *Area sampled:* The total area sampled per transect is 0.5 m^2, and the total area sampled per site is 4.5 m^2. The area sampled per composite sample is variable based on the distribution of habitats sampled at the site.

5) *Replication:* There are no site replicates collected; however, there are QA/QC replicates whereby a different team samples the same site and next year revisits at several sites.

6) *Subsampling and enumeration:* Random subsampling to 300 organisms.

7) *Taxonomic level:* Identification of all organisms to the lowest possible taxon, usually to genus, species, or species group (including Chironomids and Mites).

8) *Quality assurance procedures:* Not available.

9) *Data analysis/Metrics:* Not available.

10) *Habitat assessment:* See Lazorchak et al. 1998.

11) *Purpose for monitoring:*
    - Evaluate and improve EMAP concepts for state and local use
    - Assess the applicability of EMAP indicators at differing spatial scales, and
    - Demonstrate the utility of EMAP for resolving issues of importance to EPA Regions and states.

University of California Sierra Nevada Aquatic Research Laboratory (SNARL)

1) *Habitat selection:* Only riffle habitat is sampled within a 150 m study reach.

2) *Sampling gear:* The primary sampling gear used to collect samples is a D-frame kicknet with 250 µm mesh netting.

3) *Sampling method:* Five riffles are selected from a random number table along the 150 meter reach. The D-net is used to collect kick samples at ¼, ½ and ¾ of the stream width (always start at the location furthest downstream and work up). Kick an area approximately 30 square centimeters directly above the net (a square area with sides equal to net width) is kicked to disturb the substrate and dislodge organisms. The kicking is maintained for a count of about 10-15 seconds, then the rocks are scrubbed by hand for an additional 10-15 seconds (total 20-30 seconds at each of 3 positions = 1-1.5 minutes). Large rocks or wood debris are removed after washing them in the current into the net following each sample position. For streams less than 1-2 meters wide, the 3 kick samples are taken from both sides and middle above or singly one above another at the random number location (instead of taking all 3 across the stream when widths are greater than 1-2 meters). Because the focus of the method is on sampling across different microhabitat types in the stream including varied depth, current, substrate types – the three composited samples should represent the variety of habitat present. One or two composites may be taken if samples are dense with debris.

   When sampling in pools, only a single collection is taken within the tail zone of the pool (i.e. downstream third of pool zone) by sweeping or brushing the sample area into the mouth of the net. The net is sometimes used to scoop through sample area after the sweep. More than a single area sampled usually produces too much sample volume to process and preserve.

   The net should be quickly dipped into the stream to consolidate the material to the bottom of the D-net. Any remaining large debris is removed. The net is inverted into a bucket with 1/4 to 1/3 full of water. The net is shaken out to collect all the debris and insects. The net is dipped into the stream again to consolidate remaining contents and the net is then inverted into the bucket.

   Lighter material is elutriated with a swirling motion into the other bucket five times. Only a small volume of water is used in each elutriation so the receiving bucket does not overflow. Only rocks and sand should be left in the original bucket. These rocks are emptied into a shallow white pan (or the bottom of the bucket is closely examined). Cased caddisflies/snails are examined for and added to sample if found.

   The debris is then strained through a fine mesh aquarium net supported on one bucket (this may also serve as an elutriation since some sand will have gotten into this debris). The contents of the aquarium net is emptied into a sample container. BioQuip forceps are used to scrape any remaining debris into vial. The container is filled with ethanol to preserve the bugs, and a small volume of rose bengal stain is added.

4) *Area sampled:* The total area sampled per composite is 0.27 m^2, and the total area sampled per site is 1.28 m^2.

5) *Replication:* Five replicate composite samples are collected from each site.

6) *Subsampling and enumeration:* Random subsampling to 300 organisms.

7) *Taxonomic level:* Identification of all organisms to the lowest possible taxon, usually to genus, species, or species group (including Chironomids and Mites).

8) *Quality assurance procedures:* See website for detailed information; http://www.swrcb.ca.gov/rwqcb6/QAPP/QAPP_Index.htm

9) *Data analysis/Metrics:* See website for detailed information; http://www.swrcb.ca.gov/rwqcb6/QAPP/QAPP_Index.htm

10) *Habitat assessment:* 15 transects are spaced at 10 meter intervals along the 150 meter delineated reach length (starting at 0). Bank and channel features are measured (wetted perimeter width, bank cover category, bank angles, and vegetation cover (using densiometer) across each transect and at 5 equal-spaced points within each transect the depth, current velocity (60% depth), and substrate type (size class) are measured. Location of each site (mid-reach) is determined with a GPS unit, and elevation determined from map location (and/or barometer). Slope is measured using a hand-held leveling scope sighted on a stadia rod over a series of intervals over the 150 meter reach length. Sinuosity is determined from the ratio of reach length to minimum linear distance from the bottom to top points of the reach. Percent riparian canopy cover by type (within 1 meter on the bank) is visually estimated for the reach. Temperature, pH, conductivity and turbidity are measured using calibrated field meters. Dissolved oxygen is determined in the field using a standard test kit. Alkalinity, nitrogen, phosphate, and hardness are measured in the lab from field samples. General types of algae present are noted for each reach (algae samples from rock surfaces are also collected and preserved). Photo documentation of each reach is also made at 4 points: mid-stream looking upstream at 0, 50, 100, and at 150 meters looking downsteam.

    <u>Reach and Riffle-Pool Delineation</u>
    The first step in description of physical habitat is delineation of the 150 meter length of the stream reach along an approximation of the thalweg of the channel. To the extent possible, this measurement should be made by following along the bank contours of the channel, laying out the meter tape (50 m on a reel). This may require crossing the channel or even walking in the stream if bank vegetation cover is too dense – but this should be kept to a minimum to avoid disturbance of benthic habitat. For each 25 meter length a flag should be placed to serve as a monument for marking locations and later measurement of gradient. Over the 150 meter reach delineation, the primary data to be recorded is the position along the meter tape (to the nearest meter) where erosional and depositional habitat types begin and end – riffles and pools, respectively. This data provides an indication of the distribution and length of these major geomorphic units within each reach. The position of these habitat features will also be used to determine where the benthic invertebrate samples are to be taken by using a random number table (0-150) to assign a riffle or pool location to be sampled. Any habitat not assigned to the riffle-pool categories may be regarded as transitional glide or run habitat type. Depending on the criteria for reach selection, the starting point of a reach may be

established to maintain the reach within a certain zone defined by the problem of interest, the gradient, vegetation cover, or accessibility. Selection may also be random, using preliminary map information on the target area.

<u>Bank and Channel Features</u>
Bank features on each transect are identified according to bank cover categories (substrate type, vegetation present and eroded, stable or incised). The intersect of interest is between the water level and an approximation of the bank full height of the channel. Bank angle is also rated categorically as shallow (less than 30 degrees), moderate (30-90) or undercut (>90). Riparian vegetation cover over and next to the channel is determined using a concave mirror densiometer, taped to view the canopy in the facing direction of the measure. There are 17 grid points and vegetation reflected at those grid points is recorded at the left and right banks, and mid-stream facing up- and downstream.

<u>Transect Measures</u>
After measuring stream width (wetted perimeter), the transect is visually divided into 5 equally spaced points (visualize the mid-point as 3, and equally divide the left and right sides into points 1 and 2 and points 4 and 5). At each point, the depth and substrate type at the point of contact are recorded (recorder on bank) using a meter stick. Substrate types are grouped by size class for the mineral type, and also according to algal, vegetation or detrital components present at the point. At 60% depth the current velocity is also measured at each point (also record current meter type used and units). Discharge is calculated later for each of the 5 cells measured (current x cross-section area). Any cobble encountered is also rated according to the volume of rock embedded by fine / sand substrates (a visual estimate, calibrated among observers).

<u>Overall Reach Features</u>
The gradient of the channel is measured using a hand-held leveling scope (5X magnification) to sight off a 5 meter leveling rod. The observer serves as the tripod and so should find a position where both upstream and downstream position of the rod can be clearly observed without moving except to turn the upper body. Most readings will be taken over 25 meter intervals but where possible should be taken over 50 meter intervals to save time. The sum difference in up-down readings over 150 will give the percent slope or gradient. The sinuosity of the channel is measured as the ratio of the 150 meter thalweg stream length to the direct line distance from the top to bottom flags defining the reach. This is done by sighting to the leveling rod held at one end of the reach and walking a direct line of sight to the rod, measuring distance with a reel tape over the distance (a person to hold the tape end facilitates the several walks needed to measure the full distance). Riparian vegetation cover is visually estimated as morphological categories of cover (grass, bush, tree) and type. This provides another measure of shading, riparian development and potential inputs. Algae type present is also qualitatively scored. Notes should also be kept on any aquatic vegetation present.

11) *Purpose for monitoring:*
- Biocriteria development and assessment & monitoring.
- Livestock grazing stream restoration

- Acid Mine Drainage stream restoration monitoring.
- TMDL development for sediments.
- Reference condition sampling